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PRINCIPAL INVESTIGATOR: Amy Rowat

CONTRACTING ORGANIZATION: University of California, Los Angeles
Los Angeles, CA 90095

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Amy Rowat

E-Mail: rowat@ucla.edu

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University of California, Los Angeles
610 Charles E Young Drive South
Los Angeles, CA 90095

Cedars-Sinai Medical Center
8700 Beverly Boulevard
Los Angeles, CA 90048

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14. ABSTRACT

Treatment of high grade serous ovarian is initially effective in reducing the growth tumors, but cancer recurs in over 80% of ovarian cancer patients because cells become resistant to common, platinum-resistant chemotherapy drugs. There is a critical need for new drugs that target platinum-resistant cancer cells. We recently discovered that platinum-resistant ovarian cancer cells are more deformable than their drug-sensitive counterparts. We hypothesized that we could identify novel compounds that selectively target drug-resistant ovarian cancer cells by screening cells against libraries of small molecules using the novel Parallel Microfiltration (PMF) screening technology that we recently invented. In this first funding period, we have successfully advanced and integrated the PMF technology into the Molecular Shared Screening Resource at UCLA, thereby establishing the first facility for mechanotype screening. We have designed and optimized conditions for the mechanotype screen. Our proof-of-concept screen of cisplatin-resistant ovarian cancer cells against the Library of Pharmacologically Active Compounds (LOPAC) reveals several lead compounds. The pending studies will validate the effects of the lead compounds on cisplatin-resistant ovarian cancer cells, including cellular and molecular analyses.

15. SUBJECT TERMS

ovarian cancer, cell mechanical properties, cell mechanotype, drug discovery

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1. INTRODUCTION:

Platinum resistance is the main cause of ovarian cancer-associated mortality. There is a critical need for new anti-cancer drugs to combat drug resistant and recurrent cancers. We propose that cancer-specific alterations in cell mechanical phenotype, or mechanotype, can be an alternative target for novel therapeutic agents. The altered mechanotype of cancer cells is an emerging biomarker that can enable more accurate diagnoses, which complement existing methods used by pathologists. We previously showed that cisplatin-resistant cells exhibit characteristics of mesenchymal-type cells (Qi et al, *Nat. Sci. Reports*); cytoskeleton reorganization and epithelial-to-mesenchymal transition (EMT) are features associated with cancer progression and metastasis, as observed in many *in vitro* models of drug resistance, as well as in patients. To enable screening based on cell mechanotype, we recently invented Parallel Microfiltration (PMF) that allows simultaneous measurements of cell mechanotype. **The goal of this project is to identify compounds that reverse the mechanotype of soft, platinum-resistant cells and are effective as anti-cancer agents against these drug resistant cells**. More broadly, this study will validate the use of mechanotyping as a complementary screening method to identify compounds with efficacy as anti-cancer agents that target platinum-resistant cells. Ultimately, identifying novel molecules that modulate mechanotype, reduce metastasis of platinum-resistant cancers, and could be administered to patients, would enable effective treatment strategies for patients that have resistant subtypes and improve patient survival.

2. KEYWORDS: ovarian cancer, cell mechanical properties, cell mechanotype, drug discovery

3. ACCOMPLISHMENTS:

- **What were the major goals of the project?**

Specific Aim 1: To identify molecules that reverse the softer mechanotype of platinum-resistant cells (Specified in proposal)	**Timeline**	**Site 1: UCLA**	**Site 2: Cedars-Sinai**	**Percent completed**
Subaim 1A: PMF-screen platinum-resistant ovarian cancer cells against small molecule libraries				
Major Task 1 - Demonstrate PMF function in the MSSR	Months			
Subtask 1 - Establish readout using plate reader	1	Rowat		100%
Subtask 2 - Fabricate PMF devices that interface with liquid handlers	1-6	Rowat		100%
Milestone #1 Achieved - Replicate filtration behavior of cisplatin-resistant OVCAR (CisR) cell lines plus positive control (taxol treatment)	6			100%
Subtask 3 - Establish standard deviation of PMF in MSSR: well to well, row to row, and plate to plate variability	7-9	Rowat		100%
Milestone #2 Achieved - Define threshold above which we define a 'hit'	9			100%
Major Task 2 - Conduct screen of LOPAC collection (1280 approved drugs)				
Subtask 1 - PMF validation screen	7-9	Rowat		100%
Subtask 2 - Validate hits from initial screen using orthogonal transwell migration assay	9		Karlan/ Lawrenson	0%
Milestone #3 Achieved - Verification of hits that are known cytoskeletal-targeting drugs	9			100%
Major Task 3 - Conduct screen of chemically diverse ChemBridge and Prestwick libraries (>30,000 compounds)				

Subtask 1 - PMF screen	10-12	Rowat		80%
Subtask 2 - Generate ranked list of hits	11-12	Rowat		0%
Milestone #4 Achieved - Identification and ranking of compounds for secondary tests	12			0%
***Subaim 1B*: Validation of lead compounds by PMF**				
Major Task 4 - Validate lead hits				
Subtask 1 - Validation of hits using orthogonal (invasion) assay with the original cell lines used for the screen and independent (Kuramochi, Ince) cell lines	10-12		Karlan/ Lawrenson	0%
Subtask 2 - Perform dose response experiments using PMF with the original cell lines used for the screen and independent (Kuramochi, Ince) cell lines	10-12	Rowat		0%
Major Task 5 - Rank validated compounds based on specificity for platinum-resistant cells				
Subtask 1 - Determine IC_{50} values for platinum-sensitive (control) and -resistant (target) cells; calculate therapeutic index (TI)	12		Karlan/ Lawrenson	0%
Subtask 2 - Generate ranked list of validated compounds with specificity for platinum-resistant cells, TI < 5	12	Rowat	Karlan/ Lawrenson	0%
Milestone #5 Achieved - Identification of compounds that target platinum-resistant cells; 10 lead compounds will advance to functional studies	12			0%
Specific Aim 2: To characterize the anti-cancer potential of lead compounds using functional assays (Specified in proposal)				
Major Task 6 – Perform functional assays to investigate effect of lead compounds				
Subtask 1 - Cell cycle analysis	13-16		Karlan/ Lawrenson	0%
Subtask 2 - Cytotoxicity assays	13-16		Karlan/ Lawrenson	0%
Subtask 3 - Measure protein and mRNA levels	13-16		Karlan/ Lawrenson	0%
Subtask 4 - Determine subcellular structure	13-16		Karlan/ Lawrenson	0%
Subtask 5 - Anchorage-independent growth	13-16		Karlan/ Lawrenson	0%
Subtask 6 - Conduct studies on primary patient-derived cells	15-19		Karlan/ Lawrenson	0%
Milestone #6 Achieved - Identification of anti-cancer compounds for platinum-resistant cells	19			0%
Major task 7 - Prepare and publish manuscript on high throughput mechanotype screening to identify novel anti-cancer compounds that target platinum-resistant cells				
Subtask 1 - Prepare figures and write results section	20-21	Rowat	Karlan/ Lawrenson	25%
Subtask 2 - Write Introduction and Discussion	21-22	Rowat	Karlan/ Lawrenson	25%

Subtask 3 - Submit paper	23	Rowat	Karlan/ Lawrenson	0%
Subtask 4 - Respond to reviewer comments and resubmit	>23	Rowat	Karlan/ Lawrenson	0%
Milestone #7 Achieved - Paper accepted for publication	>23			0%

○ **What was accomplished under these goals?**

1) Major activities

Our activities focused on integrating the parallel microfiltration (PMF) system into the core high throughput screening facility at UCLA, the Molecular Shared Screening Resource (MSSR). Due to technical hurdles in the integration process, and reduced effort of the lead Graduate Student Researcher working on the project for medical reasons, we have not yet conducted the mechanotype screen but the PMF system is now successfully working in the MSSR and the final preparations are being made to conduct the screen.

2) Specific objectives

We have fully optimized the PMF system in the MSSR high throughput screening facility. After performing the screen, lead compounds will be prioritized for follow up studies to determine their effects on invasion, proliferation, cytoskeletal structure and protein expression, using both established ovarian cancer cell lines as well as cells from patient ascites.

3) Significant results and key outcomes

PMF integrated into the Molecular Shared Screening Resource. The PMF device is now integrated into the high throughput screening facility at UCLA (Fig 1). An automated pipettor is used to deliver drugs to cells in multiwell plates; after the 24 h incubation period, the drug-treated cells are lifted off the substrate into suspension. The cell suspensions are then transferred into the PMF device, which is placed in the pressure chamber. Pressure is applied to drive the suspension of cells and media through the porous membrane. The resultant cell suspension that is retained in the top well is then transferred to a 96-well plate, and placed in a plate reader to determine absorbance. Standard plate reader software is used to determine absorbance readings; wells that have a high absorbance reading, thereby indicating increased retention, and identify 'hits'.

PDMS-PMF device for high throughput screening. To achieve high throughput screening, we developed a v2 PMF device that has an array of 96 filtration units that are fabricated in a polydimethylsiloxane (PDMS) membrane using soft lithography (Fig 2C-E). The filtration units each contain an array of pillars with a gap size of 5 – 10 μm; the prototype device uses polycarbonate membranes that have similar pore sizes (Qi et al, *Nat Sci Reports*, 2015). Similar to the prototype device, air pressure is applied to drive an array of 96 individual cell samples through the array of posts; the filtrate is collected in the bottom wells and the volume, which is measured using a plate reader, reflects the deformability of the cells. A higher filtrate volume indicates the cells are more deformable,

Figure 1. **PMF setup in the Molecular Shared Screening Resource**, UCLA's high throughput screening core facility. Photo shows 96-well pipette head loading samples into the PMF device top plate.

while a lower filtrate volume reflects a sample of cells that are less deformable and tend to occlude the gaps between pillars. We proposed to use the v2 PDMS-PMF device as these can be rapidly fabricated, enable precise control of the gap sizes, and can interface with existing high throughput screening equipment (Fig 2B), including plate readers that make the assay readout more efficient.

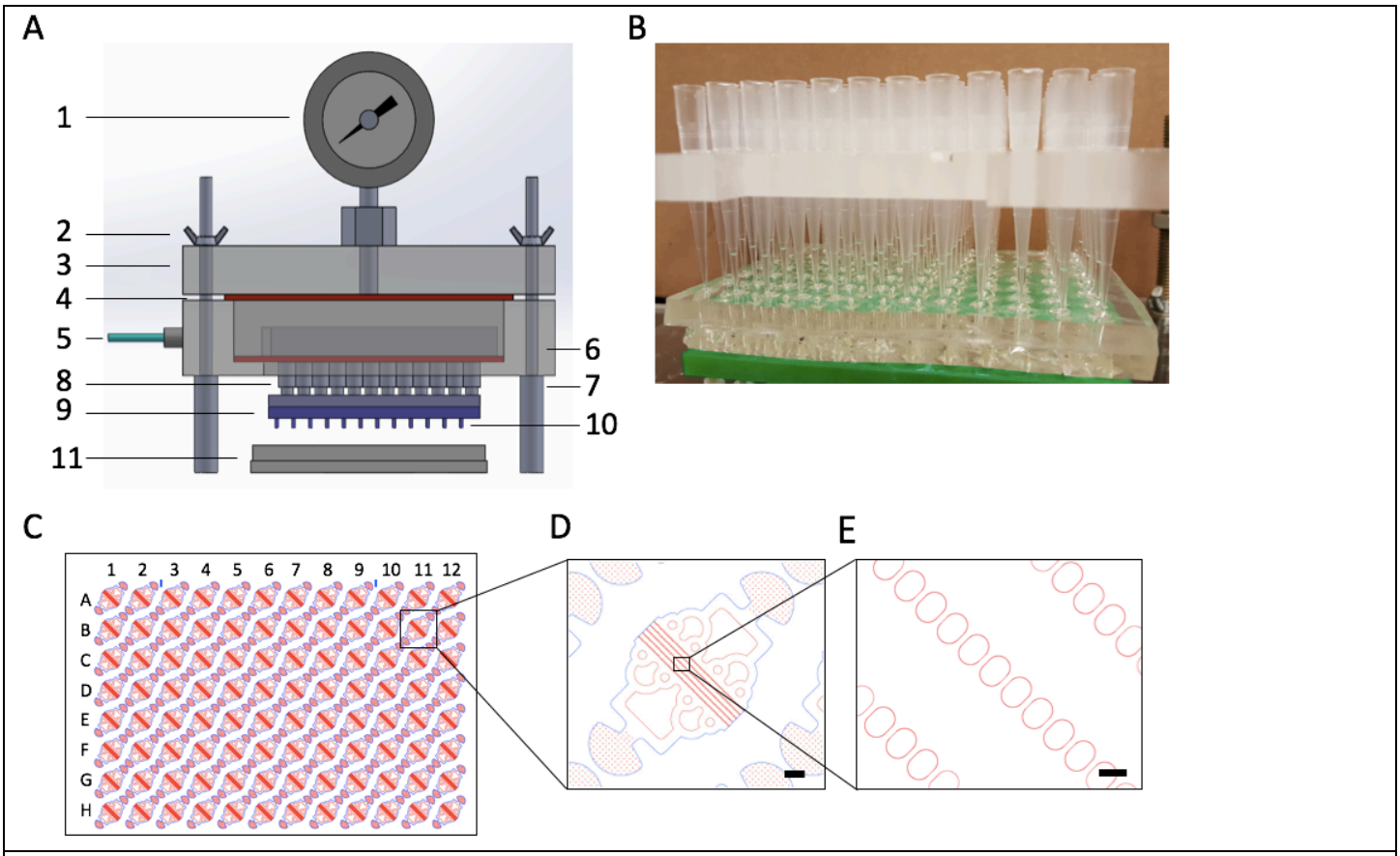

Figure 2. **PDMS-PMF device**. (A) Schematic of PDMS-PMF device. 1. Pressure gauge. 2. Wing nuts for assembling. 3. Pressure chamber. 4. Rubber sealing pad. 5. Tubing that connects to compressed air (pressure source). 6. Custom-fabricated plate holder. 7. Supporting pillars. 8. 96-well plate with tubing. 9. PDMS-membrane that contains array of 96 individual microfluidic devices. 10. Outlet tubing. 11. Collection (96-well) plate. (B) PDMS-PMF device aligns with a standard 96-well pipette array. (C) Plan view of array of 96 microfluidic devices in the PDMS membrane. (D) Single filtration device. Scale, 1 mm. (E) Micropillars with defined interpillar gap size through which cells are filtered. Scale, 100 μm.

Establish readout using plate reader. To achieve high throughput measurements of filtration, we use a plate reader to measure the absorbance of the filtered cell media at 560 nm, which is the peak absorbance of phenol red (Fig 3). We determine the standard curve by measuring the absorbance of known volumes of media to backout the filtrate volume based on absorbance readings in a 96-well plate.

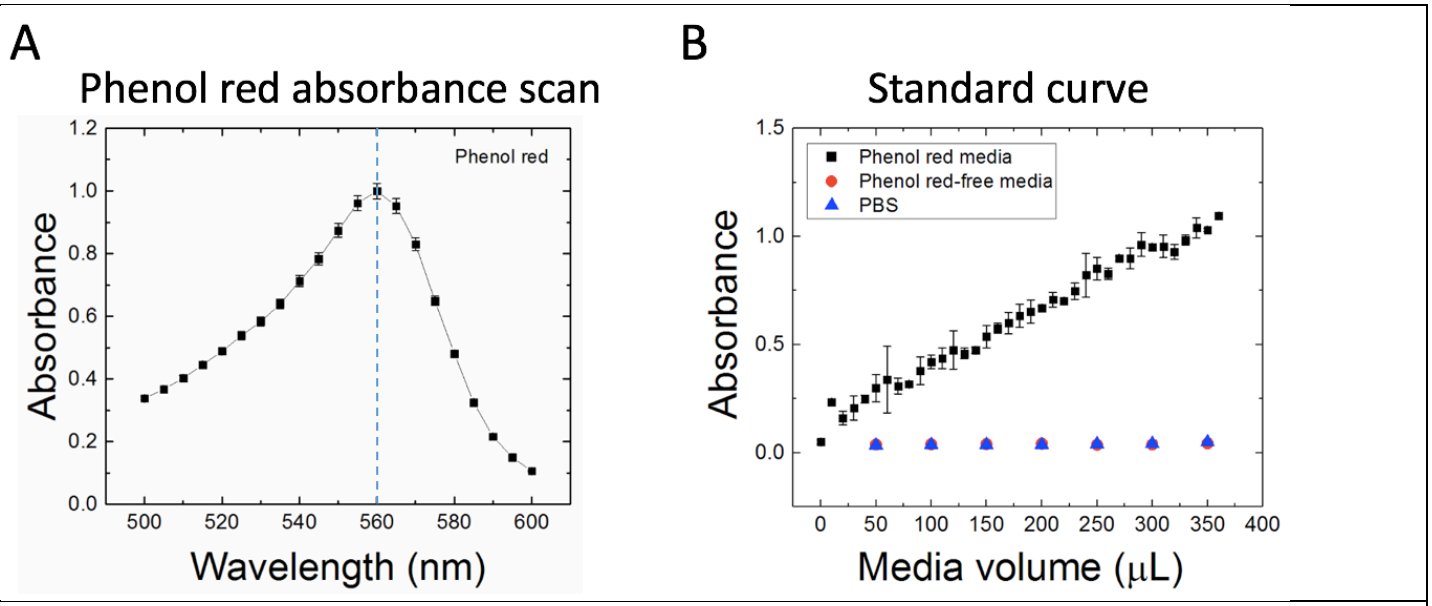

Figure 3. Calibrating the plate reader to use absorbance as a metric for volume of media filtered. We detect absorbance at 560 nm, which is the peak absorbance of phenol red. (A) Standard curve is obtained by determining the absorbance for known volumes of media. Dashed line indicates the peak absorbance at 560 nm. (B) Standard curve. Data points show average over 3 independent experiments. Error represents standard deviation.

Replicate filtration behavior of cisplatin-resistant OVCAR (CisR) cell lines plus positive control (paclitaxel treatment). We next confirmed that we could detect the difference in deformability between the paclitaxel treated-OVCAR5-Cis-R cells (positive control) versus DMSO-treated (control) cells. As shown in Fig 4, we achieve a statistically significant difference in absorbance between OVCAR5-Cis-R cells treated with 10 μM paclitaxel compared to DMSO control. These results are consistent with the decreased deformability of the paclitaxel-treated Cis-R cells that we previously observed using the prototype PMF device.

While we observe similar filtration behavior of the Cis-R versus positive control sample using the PDMS-PMF device, we also rigorously characterized the device to determine the effects of cell density in the loading sample versus in the filtrate; this revealed that in the working density range of 0.4 to 0.5 million cells/mL, there is a linear dependence of cell density, indicating there is no separation of cells occurring in the range of cell densities used in experiments (Fig 5A). We also tested a range of gap sizes to achieve optimal dynamic range, and determined a 10 um gap size results in a larger statistically significant difference in absorbance for CisR versus CisS cells (Fig 5B). Finally, we conducted filtration tests across varying applied pressure and times to determine the optimal settings for the screen (Fig 5C,D). In addition, we optimized drug treatment conditions using the paclitaxel positive control (Fig 6).

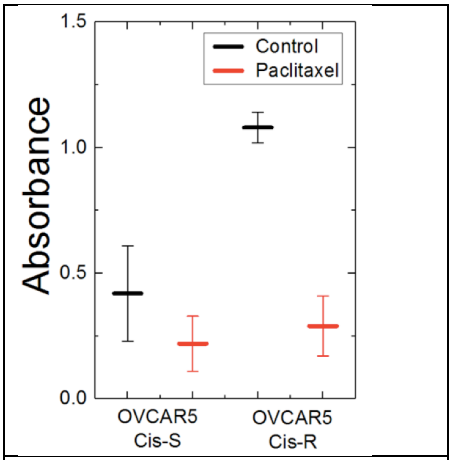

Figure 4. Cisplatin-resistant OVCAR5 (CisR) treated with paclitaxel have a higher absorption reading, indicating there is a larger filtrate volume that results from cells that are less deformable than the non-treated control cells. Data points show average absorbance readings from four wells across three independent experiments.

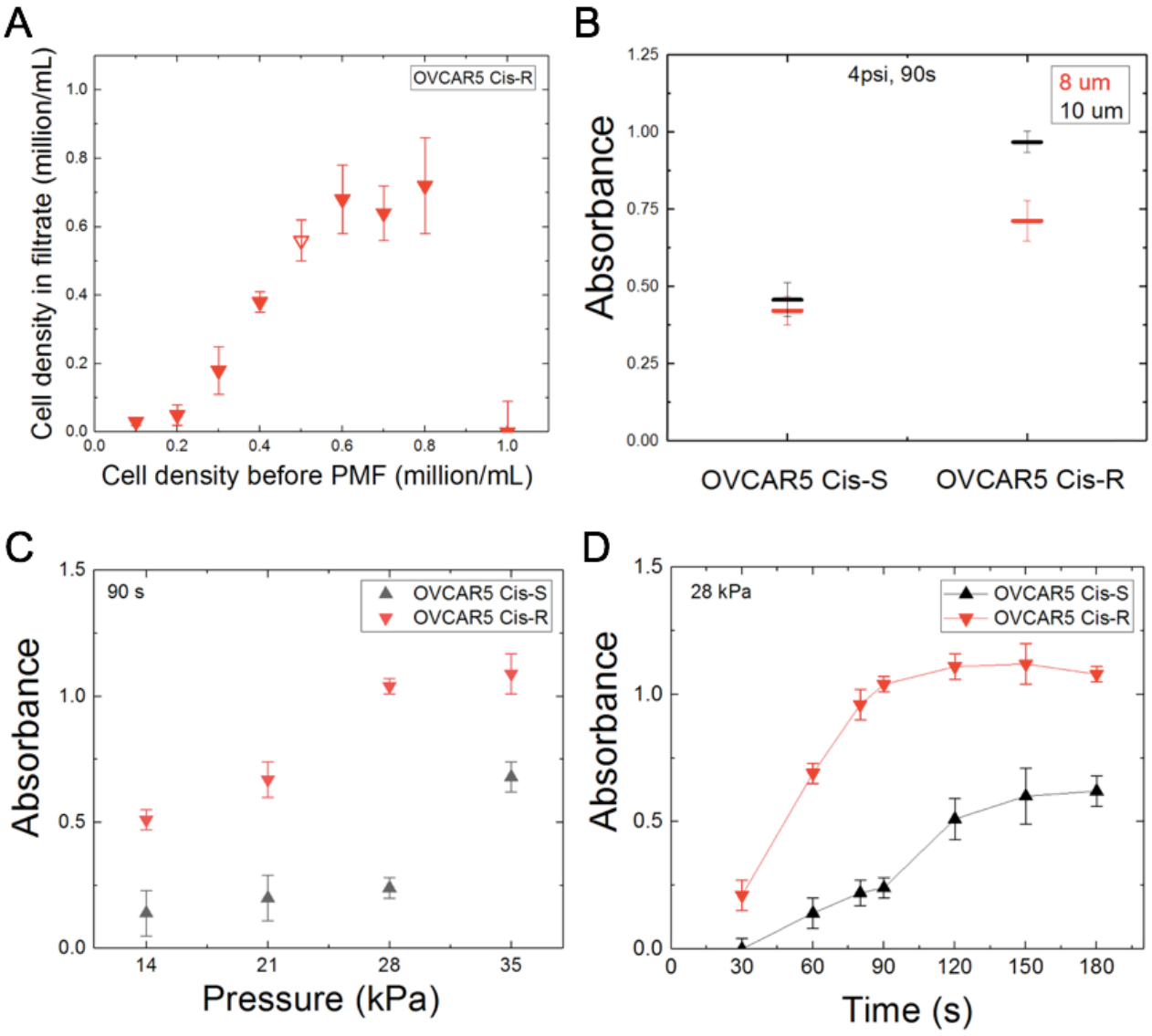

Figure 5. Optimization of conditions for the PDMS-PMF device in the MSSR: (A) Comparison of cell densities in the collected filtrate after the measurements and in the inlet sample before the PMF. (B) Absorbance of filtrate for different gap sizes for cisplatin-sensitive (Cis-S) versus –resistant (Cis-R) cells. (C) Pressure sweep for optimization of driving pressure for filtration of OVCAR5 Cis-S versus Cis-R cells. (D) Time dependence of filtrate absorbance for OVCAR5 Cis-S versus Cis-R cells. Data points show average absorbance readings from four wells across at least two independent experiments.

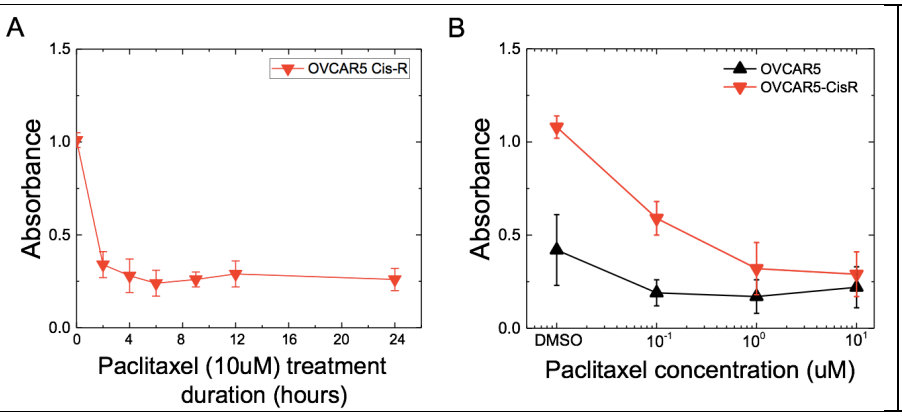

Figure 6. Optimization of treatment conditions for paclitaxel-treated cells filtered in the PDMS-PMF device. (A) Treatment time; and (B) Paclitaxel concentration. Data points show average absorbance readings from four wells across at least two independent experiments.

Establish standard deviation of PDMS-PMF device in MSSR. Despite rigorous optimization, the variability filtration from well-to-well using PDMS-PMF device exceeded the threshold for conducting a high throughput screen (Fig 7), which is indicated by the unacceptable z-factors that are below 0.3 (Fig 8) when comparing the filtration of OVCAR5-CisR cells treated with DMSO versus paclitaxel control. We hypothesized that the variability between wells could result from variation in fluidic resistance through the array of 96 individual devices; this could stem from small differences in device geometry that occur during PDMS device fabrication, the presence of air bubbles, and/or clogging due to particles of PDMS or other debris that may alter the flow properties of individual devices. We found that surface treatment using bovine serum albumin (BSA) or Pluronics F-127 prior to filtration had no significant effect (data not shown). The development of a PDMS-PMF device that exhibits reduced variability that is required for high throughput screening will be the topic of future studies. Meantime, the results of our rigorous optimization provide the basis for a manuscript that we will submit for publication that describes the novel concept of the PDMS-PMF device (Gill et al, Manuscript in preparation).

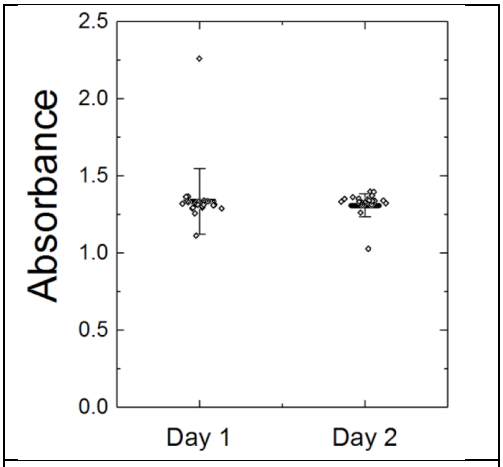

Figure 7. Dissecting the origins of variability in the volume of filtered media between devices. To illustrate day-to-day variability, we show data collected on two independent days.

Device type	Conditions tested	Cell type	PMF settings	Z-factor
PDMS-PMF	10 µM paclitaxel treatment, 0.5 million/mL cell density, 8 µm pillar gap size, 360 µL sample volume, absorbance measurement	OVCAR5-Cis-R	4 psi 90 s	-0.36
PDMS-PMF	10 µM paclitaxel treatment, 0.5 million/mL cell density, 10 µm pillar gap size, 360 µL sample volume, absorbance measurement	OVCAR5-Cis-R	4 psi 90 s	0.2
PDMS-PMF	10 µM paclitaxel treatment, 0.5 million/mL cell density, 10 µm pillar gap size, 360 µL sample volume, absorbance measurement	OVCAR5-Cis-R	5 psi 60 s	-0.56
PDMS-PMF	10 µM paclitaxel treatment, 0.5 million/mL cell density, 10 µm pillar gap size, 360 µL sample volume, cell count measurement	OVCAR5-Cis-R	4 psi 90 s	-0.9
PDMS-PMF	10 µM paclitaxel treatment, 0.2 million/mL cell density, 10 µm pillar gap size, 360 µL sample volume, absorbance measurement	OVCAR5-Cis-R	3 psi 180 s	-1.35
PDMS-PMF	10 µM paclitaxel treatment, 0.2 million/mL cell density, 10 µm pillar gap size, 360 µL sample volume, cell count measurement	OVCAR5-Cis-R	3 psi 180 s	-0.7
Small volume prototype	10 µM paclitaxel treatment, 0.5 million/mL cell density, 10 µm membrane, 180 µL sample volume, volumetric measurement	OVCAR5-Cis-R	1 psi 60 s	-2.7
Modified-PMF	10 µM paclitaxel treatment, 0.5 million/mL cell density, 10 µm membrane, 350 µL sample volume, volumetric measurement	OVCAR5-Cis-R	0.3 psi 30 s	0.3
Modified-PMF	10 µM paclitaxel treatment, 0.5 million/mL cell density, 10 µm membrane, 350 µL sample volume, Absorbance measurement	OVCAR5-Cis-R-H2B-BFP	0.2 psi 20 s	0.25
Modified PMF	10 µM paclitaxel treatment, 0.4 million/mL cell density, 10 µm membrane, 350 µL sample volume, volumetric measurement	OVCAR5-Cis-R-H2B-BFP	0.2 psi 15 s	0.3

Figure 8. Z-factor results obtained for the PDMS-PMF device. Despite rigorous optimizations to reduce variability of the PDMS-PMF device, a z-factor of only 0.1 was achievable; a value of $0.3 < z < 0.5$ is minimally required for a meaningful screen; $0.5 < z < 1$ is optimal for identification of statistically robust hits. Our current device optimization, we achieve $z = 0.3$, and with additional replicates we plan to achieve a higher z-factor prior to the screen. OVCAR5-Cis-R-H2B-BFP denotes the genetically modified OVCAR5-Cis-R cells that stable express the blue fluorescent protein (BFP) labeled histone 2B (H2B); this facilitates automated cell counting during the screen to account for any variation in cell densities.

○ **What opportunities for training and professional development has the project provided?**

The project is providing an excellent training opportunity for graduate student researcher in Molecular, Cellular, and Integrative Physiology (MCIP), Navjot Kaur Gill. Working on this challenging multidisciplinary project, Ms. Gill has developed her unique skill set in cancer biology and biotechnology in establishing this novel mechanotyping assay in the Molecular Shared Screening Resource (MSSR). She has worked closely with PI Rowat to troubleshoot the integration and optimization of the PMF technology in the MSSR. Ms. Gill also works closely with Dr. Robert Damoiseaux, Scientific Director of the MSSR to integrate PMF into the MSSR and implement the mechanotype screen. She has therefore gained valuable skills in assay development and laboratory automation. In addition, Ms. Gill is gaining valuable knowledge in ovarian cancer biology through the collaboration with Dr. Rao and Cedars Sinai. She is involved in the design of orthogonal experiments to test the effects of lead compounds on the invasion and proliferation of ovarian cancer cells, including patient cells.

Ms. Gill is the first author on a manuscript on the PDMS-PMF platform that we will submit for publication in the coming months. She is also the first author on the protocol describing PMF methodology that is published on the Nature Protocol Exchange. Ms. Gill has also presented her work in a seminar for the MCIP graduate program on 07/11/17, entitled "*Cell mechanotype in tumor progression and metastasis*".

○ **How were the results disseminated to communities of interest?**

PI Rowat delivered the following talks describing the project and acknowledging DoD funding:

• Cedars-Sinai Medical Center, Cancer Biology Seminar

• University of Houston, Physics Colloquium

• Mechanobiology Institute & Biophysical Society Thematic Meeting on '*Mechanobiology of Disease'*, Singapore

• University of San Diego, Jacobs School of Engineering Symposium on '*Putting together the Cell Mechanome'*

○ **What do you plan to do during the next reporting period to accomplish the goals?**

In the next reporting period, we will produce a prioritized list of lead compounds that are identified from the PMF screen. Compounds will be assessed for their anti-cancer effects on cisplatin-resistant cells, including proliferation, invasion, and ability to form tumor spheroids. Effects on patient cells from ascites will also be determined.

4. IMPACT:

What was the impact on the development of the principal discipline(s) of the project?

PMF-mechanotype screening provides a new paradigm for high throughput screening. Existing methods for measuring cell deformability rely on sequential measurements of cells. The ability to simultaneously measure the deformability of cell samples in a multiwell plate format enables scale-up of deformability assays. This is a key step towards advancing the use of mechanotype in clinical and research applications that require high throughput studies, such as in the context of small compound screening.

○ **What was the impact on other disciplines?**

The ability to screen cells based on mechanotype opens up possibilities for screens that are relevant to other fields from cell biology to cancer. For example, a shRNA-screen could identify the origins of nuclear shape stability and mechanotranduction.

○ **What was the impact on technology transfer?**

The PMF mechanotype-screening is now established in the UCLA Molecular Shared Screening Resource (MSSR). This core facility is available to UCLA and external researchers, who will now have the possibility to conduct mechanotype screen. Unexpectedly we discovered that the PMF system can enable washing of cells that are larger than the pores of the membrane in a high throughput setting as it interfaces with the automated liquid handling platform.

○ **What was the impact on society beyond science and technology?**

Nothing to Report.

5. CHANGES/PROBLEMS:

Actual or anticipated problems or delays and actions or plans to resolve them

We experienced a delay in our project plan due to the technical hurdles in scaling up the PMF technology to integrate with existing high throughput facilities. The original plan was to develop a polydimethylsiloxane (PDMS-PMF) microfluidic device with an array of 96 filtration units, which could interface with existing high throughput equipment. While we successfully fabricated this device, and built the requisite hardware (e.g. pressure chamber, tubing connections) to interface with arrays of pipettes and 96-well plates, the measurement variability exceeded the threshold required to robustly identify hits: we determined that the PDMS-PMF device can achieve a z-factor of only 0.1; at minimum, $z > 0.3$ is required for a high throughput screen, and $0.5 < z < 1$ represents an excellent assay. We attempted to improve the z-factor for the screen using this device (Fig 8), by optimizing various factors including: cell density; applied pressure; and F-127 and BSA surface treatments of the devices. However, it was not possible to achieve improved z-factors by tuning any of these experimental parameters.

To achieve the goal of identifying small molecules that cause cisplatin-resistant cells to be stiffer, and also have anti-cancer effects, we adapted the prototype PMF device into the MSSR. With this modified-PMF device we achieve a z-factor of 0.3 for the screen of cisplatin-resistant ovarian cancer cells with paclitaxel-treated positive control. The prototype PMF device is now successfully integrated into the MSSR, however, the prototype PMF device limits the number of automated steps in the workflow, and thus reduces assay throughput for the following reasons:

- To achieve optimal resolution between positive control (paclitaxel-treated) and DMSO-treated cells requires each well to have 0.4 cells/mL. To achieve this number of cells after drug treatment, washing, and liftoff into suspension requires culturing cells in 24-well plates that have larger surface area than 96-well plates; this requires additional cell culture to obtain the number of cells required for the specified cell density per well.
- Since 96-well plates are required for the high throughput readout using a plate reader, cells must be transferred from the original 24-well plates used for culture to the 96-well format for filtration and readout. We use adaptable pipette heads to achieve liquid transfer between the multiwell plates of different formats, but this requires additional time, and thus reduces throughput.
- The automated pipette heads in the MSSR cannot be used to dispense the 400 μL large volumes to 24 well plates, so these transfers are performed manually. For example, the initial placement of 350 μL media into the well, addition of trypsin post drug treatment, and wash steps.

- Steps in the device setup are also performed manually: porous membranes are placed in the PMF device, and the device is manually placed in the pressure chamber for application of positive pressure that drives the flow of cell suspensions through the porous membrane

Given these limitations in assay throughput, we have focused the screen on the Library of Pharmacologically Active Compounds (LOPAC) collection of 1280 compounds. This curated, well-characterized collection of small molecules contains pharmaceutically relevant structures annotated with biological activities, which represent major classes of compounds that impact most signaling pathways and all major drug target classes. Therefore the LOPAC library will enable us to identify the classes of compounds that target cisplatin-resistant ovarian cancer cells. Using the modified-PMF device, the LOPAC screen of 1280 compounds (16 x 96-well plates) will be complete within ~8 days. This is a major advance over existing methods that require sequential measurements, and will provide the basis for a high impact manuscript (target journal: Nature Methods, eLife). While we had initially proposed to screen a larger library of 30,000 compounds, this would require 200 days (6 months), which lies out of the scope of the Pilot Award timeline. The future development of a PDMS-PMF device with reduced variability would make it feasible to achieve more complete automation and increase assay throughput.

Addressing these experimental challenges provided the opportunity to identify ways to improve the PMF methodology for future high throughput mechanotype screening. To reduce the measurement variability, a redesign of the microfluidic device filtration units could include: eliminating the branching of channels to reduce trapped air in the device, which can contribute to variability between measurements changing the dimensions of the device to avoid dead volume in the corners; reducing the dead volume of the filtration unit, which is currently 1 μL; reducing the number of constrictions so that the device could be operated at a lower cell density in addition to the channel geometry that delivers the fluid to the constrictions to avoid bubbles. Our current device works well at loaded cell density 10 times more than what is feasible for complete automation. More rigorous optimization experiments and simulations are required to fully address this challenge, which is outside the scope of this DoD-funded project and will be tackled in future work.

Changes that had a significant impact on expenditures – N/A

Significant changes in use or care of human subjects, vertebrate animals, biohazards, and/or select agents – N/A

Significant changes in use or care of human subjects – N/A

Significant changes in use or care of vertebrate animals – N/A

Significant changes in use of biohazards and/or select agents – N/A

6. PRODUCTS:

a. Publications, conference papers, and presentations

i. <u>Journal publications</u>

Under review:

Nyberg KD, Bruce SL, Nguyen AV, Chan CK, Gill NK, Kim TH, Sloan EK, Rowat AC#. Label-free prediction of cancer cell invasion by single-cell physical phenotyping. Under review at Nature Microsystems & Nanoengineering. *Acknowledgement of federal support: Yes.*

Manuscripts in preparation:

Gill NK, Nyberg KD, Qi D, Rowat AC#. Deformability-based screening using high throughput cell filtration. (90% of data collected, draft of manuscript written) *Acknowledgement of federal support: Yes.*

Gill NK*, Nyberg KD*, Scott M, Qi D, Khismatullin DB, Rowat AC♯. Effect of single cell mechanical properties in microfiltration. (100% of data collected, draft of manuscript written) *Acknowledgement of federal support: Yes.*

ii. **Other publications, conference papers, and presentations.**

Other publications:

Gill NK, Qi D, Kim TH, Chan CK, Nguyen AV, Nyberg KD, Rowat AC. A protocol for screening cells based on deformability using parallel microfiltration. Nature Protocol Exchange. *Acknowledgement of federal support: Yes.*

Presentations:

University of San Diego, Jacobs School of Engineering Symposium on 'Putting together the Cell Mechanome' (August 2016) *Acknowledgement of federal support: Yes.*

Mechanobiology Institute & Biophysical Society Thematic Meeting on 'Mechanobiology of Disease', Singapore (September 2016) *Acknowledgement of federal support: Yes.*

University of Houston, Physics Colloquium (October 2016) *Acknowledgement of federal support: Yes.*

Cedars-Sinai Medical Center, Cancer Biology Seminar (March 2017) *Acknowledgement of federal support: Yes.*

b. **Technologies or techniques**
The mechanotype-screening platform is now installed in the UCLA core facility, the Molecular Shared Screening Resource.

7. PARTICIPANTS & OTHER COLLABORATING ORGANIZATIONS

What individuals have worked on the project?

Name:	Amy Rowat (UCLA)
Project Role:	PI
Nearest person month worked:	2.00
Contribution to Project:	Dr. Rowat oversaw all aspects of the project, including experimental design, execution, and data analysis and interpretation. She wrote the manuscript (Gill et al, Manuscript in preparation) and prepared presentations as well as the progress report.

Name:	Dr. Robert Damoiseaux (UCLA)
Project Role:	Co-Investigator
Nearest person month worked:	0.5
Contribution to Project:	Dr. Damoiseaux oversaw the integration of Parallel Microfiltration (PMF) into the Molecular Shared Screening Resource at UCLA. He also oversaw the design of the mechanotype-screen and contributed to software developments to ensure seamless integration of PMF into the high throughput facilities.

Name:	Dr. Jianyu Rao (UCLA)
Project Role:	Co-Investigator
Nearest person month worked:	0.5
Contribution to Project:	Dr. Rao contributed expertise on the use of platinum-sensitive versus -resistant carcinomas in the mechanotype-screen. His lab will also advise in Year 2 on the functional assays to characterize malignant phenotypes and drug response, including viability and apoptosis.

Name:	Dr. Beth Karlan (Cedars Sinai)
Project Role:	Co-Investigator
Nearest person month worked:	0.12
Contribution to Project:	Dr. Karlan advised on the translational aspects of the proposal and participated in experimental design.

Name:	Dr. Kate Lawrenson (Cedars Sinai)
Project Role:	Co-Investigator
Nearest person month worked:	0.12
Contribution to Project:	Dr. Lawrenson advised on the translational aspects of the proposal and participated in experimental design.

Name:	Navjot Kaur Gill (UCLA)
Project Role:	Graduate Student Researcher
Nearest person month worked:	12.00
Contribution to Project:	Ms. Navjot Kaur Gill has conducted all aspects of the project, including developments in the hardware and software for the mechanotype screen, cell culture and drug treatment optimizations, fabrication of PMF devices, as well as experimental design, execution, and data analysis and interpretation. She wrote

	the manuscript (Gill et al, Manuscript in preparation) and prepared presentations.

Has there been a change in the active other support of the PD/PI(s) or senior/key personnel since the last reporting period?

• **Dr. Rowat** – no change

• **Dr. Karlan:**

Title: *Immune Infiltrate Dynamics in Cancer Progression*
Agency: Department of Defense (W81XWH-17-1-0144)
Agency Contact: Chris Baker - (301) 619-2332
USA Medical Research, 820 Chandler Street, Fort Detrick, MD 21702-5014
Period: 05/01/17 – 04/30/20 **Funding:**
Role: Co-Investigator **Effort:** 2% - 0.24 Calendar Months
Project Goals: The goal of this project is to systematically characterize immune cell enrichment in the microenvironment of ovarian cancers and identify immune cell subsets that are prognostically relevant for patient care and/or can be used to develop more effective therapies targeting the tumor microenvironment.
Specific Aims: 1) Identify biomarkers and therapeutic targets in the tumor stroma by correlating stromal factors with clinical outcomes; 2) Generate a spatiotemporal map of stromal activity associated with tumor progression and chemoresistance; and 3) Demonstrate that targeting CAFs improves the immune response to tumors.

Title: *Clinical Proteomic Technology Assessment for Cancer (CPTAC) Program*
Agency: National Institutes of Health (HHSN261200800001E) / Leidos Biomedical Research
Agency Officer: Tara Butler - (301) 846-6722
Research Contracts, P.O. Box B, Frederick, MD 21702
Period: 10/12/16 – 09/30/17 **Funding**:
Role: Principal Investigator **Effort**: 1% - 0.12 Calendar Months
Project Goals: The overarching goal of CPTAC is to improve our ability to diagnose, treat and prevent cancer. To achieve this goal in a scientifically rigorous manner, the National Cancer Institute (NCI) launched CPTAC to systematically identify proteins that derive from alterations in cancer genomes and related biological processes, and provide this data with accompanying assays and protocols to the public.
Specific Aims: 1) Identify and characterize the protein inventory from tumor and normal tissue biospecimens; 2) Integrate genomic and proteomic data from analysis of common cancer biospecimens; 3) Develop assays against proteins prioritized in the discovery stage as potential biomarker candidates; 4) Perform testing of verification assays in relevant cohorts of biospecimens.

Title: *Cancer-Associated Fibroblasts Alter the Composition of B Cells in Solid Malignancies*
Agency: National Institutes of Health (1R01CA208753)
Agency Contact: Alania Foster - (240) 276-5375
9609 Medical Center Drive, BG 9609 MSC 9760, Bethesda, MD 20892-9760
Period: 09/02/16 – 07/31/21 **Funding:**
Role: Co-Investigator **Effort:** 2.5% - 0.30 Calendar Months
Project Goals: The goals of this project are to provide a quantitative map of B-cell subsets in primary and metastatic ovarian cancer and test the bi-directional interaction between B cells and cancer-associated fibroblasts in the tumor microenvironment. Results from this project will serve as a foundation for therapeutic targeting of the B-cell-microenvironment interface to enhance tumor immunity.

Specific Aims: 1) Detail map of individual developmental stages of B-cells in primary and metastatic OVCA; 2) Identify modulatory factors produced by CAFs as well as the developmental stages of B-cell subsets for specific therapeutic targeting; and 3) Identify potential B-cell factors capable of modulating CAFs that could be used for simultaneous enhancement of immune response and depletion of CAFs.

• **Dr. Lawrenson:**

Title: *The Role of Splice Quantitative Traits in Ovarian Cancer Pathogenesis*
Sponsor: National Cancer Institute (R21 CA220078-01)
Agency Contact: Viviana Knowles – (240) 276-5157
9609 Medical Center Dr., BG9609 MSC 9760, Bethesda, MD 20892-9760
Period: 06/15/17 – 05/31/19 **Funding**:
Role: PI **Effort:** 3%
Goal: The overarching goal of this project is to establish the contribution of differential splice variation as a functional mechanism underlying susceptibility to epithelial ovarian cancer.
Specific Aims: 1) To integrate genetic association study data form EOC cases control studies with genome-wide transcriptome profiling data to identify splice quantitative traits at risk loci; and 2) To validate the functional significance of risk associated spQTLs and the role of candidate susceptibility genes in EOC development

Title: *Functional Variation in Long Non-Coding RNAs and Ovarian Cancer Risk*
Sponsor: NIH (R01 CA207456-01) / Moffitt Cancer Center (10-18708-99-01-S1)
Agency Contact: Diana Canerday – (813) 745-6079
12902 Magnolia Drive, Tampa, FL 33612
Period: 09/01/16 – 09/30/21 **Funding:**
Role: Co-Investigator **Effort:** 2%
Goal: To identify and functionally characterize long noncoding RNAs involved in germline susceptibility to epithelial ovarian cancer.
Specific Aims: 1) Identify lncRNA SNPs associated with EOC risk and ovarian-related lncRNAs; 2) Detect lncRNAs that show differential expression in high-grade serous EOC tumors compared to normal tissues; 3) Correlate lncRNA genotype with lncRNA expression and identify lncRNA target coding genes; and 4) Evaluate the functional role of risk SNPs and candidate lncRNAs in EOC development.

What other organizations were involved as partners?

Organization Name: Cedars-Sinai Medical Center

Location of Organization: Los Angeles, CA

Partner's contribution to the project:

Collaboration: During this first funding period PI Rowat met with Co-Investigators, Dr. Beth Karlan and Dr. Kate Lawrenson, at Cedars-Sinai Medical Center to discuss research directions. Pending follow-up studies of lead compounds will involve the use of facilities and resources at Cedars-Sinai.

8. SPECIAL REPORTING REQUIREMENTS

a. **COLLABORATIVE AWARDS:** An independent report is being submitted by Collaborating PIs, Dr. Karlan and Dr. Lawrenson from Cedars Sinai Medical Center.